Collagen preparation for the controlled release of active substances

A number of polymers are used in the production of administration forms for pharmaceutical or cosmetic active substances. Depending on the site of application, these are used to impart to the respective administration form the desired properties. Agents for the treatment of wounds and implantable materials should be adapted to the surface of the respective application site, and they should not interfere with the function and activity of body cells, such as keratinocytes, fibroblasts, or endothelial cells. In these cases the spectrum of suitable polymers is therefore limited to those having an excellent compatibility on contact with connective tissue and which preferably are biodegradable.

For quite a long time now, collagen, the main protein of the connective tissue, has gained a special place among the polymers suitable for this purpose. This is due to the biological compatibility and degradability of the administration forms produced therefrom. Such collagen preparations are for the most part used as wound dressings without any additional additives. However, there have also been attempts to charge collagen matrices of most different kinds with biologically active substances and to influence the release of these substances, which in general follows the dissolution and/or enzymatic degradation of the collagen carrier matrix, by the kind, structure and composition of the matrix.

To produce collagen matrices - such as those described in, for example, US 5 024 841, US 4 789 663, US 4 774 091, US 4 563 350, US 5 246 457, or EP 429 438 - solutions of telopeptide-free, acid-soluble collagen are used in general. These are used to obtain reconstituted fibrils by means of dialysis, shift of the pH, or other processes; and these fibrils are then processed by means of different methods into preferably porous matrices. These preparations can exhibit an excellent biocompatibility since they consist of pure

collagen, but they have the disadvantage that a retardation of the active substance release is limited owing to the use of soluble collagen.

Such a release retardation can be achieved by decreasing the solubility of the collagen matrix by using cross-linking agents or binders, as is described, for example in US 4 409 332, DE 2 843 963, WO 93/00890, or WO 85/04413, or by using coating agents, e.g., described in DE 38 41 397, or by combining water-soluble collagen with other polymers, preferably natural anionic polymers or their derivatives. However, owing to the additional components, additional toxicological risks which, in particular when the known cross-linking agents for collagen are used, should not be underestimated must be accepted in each of these cases.

Another possibility of delaying the active substance release is described in EP 518 697. Here, laminates are produced from water-soluble and/or water-insoluble collagen, which consist of one or several active substance-containing reservoir layers and one layer retarding the active substance supply. As compared to the above-mentioned preparations, a retardation of the release while minimizing the toxicological risks could be achieved with such laminates. However, they have the disadvantage that their production is extremely expensive and that the adherence of the layers can only be achieved with "moist" films. Dry films which are absolutely necessary in case of active substances susceptible to decomposition cannot be joined to form such laminates, for example.

However, a retardation of the active substance release is not always desired. EP 224 453 describes a collagen matrix which mainly consists of water-insoluble, reticulated collagen and additionally comprises water-soluble, non-reticulated collagen. In cosmetic preparations, e.g., face packs, said soluble collagen serves as active substance which, after application, is dissolved out by

means of the natural cutaneous moisture or by extremely moisten-
ing the preparation which then takes effect on the skin. For phar-
maceutical purposes, active substances can be incorporated into
the preparation, these are then dissolved out and released from
the collagen matrix together with the soluble collagen very quickly
after application. Such a collagen preparation can be useful in
cases where a rapid release is desired and appropriate.

Mechanisms to delay or accelerate the active substance release
from collagen preparations have been described in many ways.
However, due to the given composition, each of these prior art
collagen preparations offers only one possibility of influencing the
release, and therefore they have a given release profile each tailor-
made to one specific problem, e.g., a certain active substance or
certain active substance group, a certain therapeutic principle, or a
certain disease. None of the prior art collagen preparations can
achieve a selective and at the same time manifold control of the
active substance release, i.e., permit a variable and individual ad-
aptation of the release kinetics of active substances to differing
problems, wherein factors, such as different active substance
properties and differences in the onset and duration of action, can
be considered adequately for each case.

Accordingly, it was the object of the present invention of find a
collagen preparation which is not only suitable for a certain active
substance, a certain active substance combination, or a certain
release profile, but also permits - for a great variety of uses - a
wide-ranging, reliable control of the active substance release
adapted to the respective problem.

Most surprisingly, the solution of this object has been found in a
collagen preparation for the controlled release of active sub-
stances, comprising mixtures of acid-insoluble collagens having
different molecular weight distributions.

According to one embodiment, the collagen preparation comprises different active substances. It may additionally comprise adjuvants, such as viscosity regulators, binders, humectants, softening agents, penetration enhancers, preservatives, disinfectants, pH-regulators, antioxidants, active substance stabilizers, oils, fats, waxes, emulsion stabilizers, odorous substances, dyes, and/or inert fillers.

According to a preferred embodiment, the insoluble collagen is telopeptide-free, native, uncross-linked Type-1-collagen. This may be an insoluble collagen which is a product obtained from calfskin by means of alkaline decomposition.

Furthermore, the collagen preparation may be present in the form of powders, dusts, microparticles, fibers, flakes, foams, sponges, needles, small rods, tablets, gels, creams, single-layer films, or laminates.

Advantageously, the collagen preparation may include combinations of different administration forms to achieve the desired release kinetics.

It is preferred that the collagen preparation be bioadhesive.

A process for the production of the collagen preparation may comprise spray drying, freeze-drying, coating or casting with subsequent drying, phase separation and coacervation processes, compression, or filling into containers.

According to the process, the active substance release can additionally be influenced and/or controlled by the mixing ratio of acid-insoluble collagens with different molecular weight distributions.

According to the process, the active substance release can additionally be controlled by dissolution, swelling, or erosion of the collagen preparation. Another possibility of controlling the active substance release is by biological degradation of the collagen preparation.

The use of the collagen preparation according to the present invention consists in the controlled release of the active substance to wounds. However, the use may also be directed to the controlled release of active substances to intact skin. Finally, the use of the collagen preparation may serve to implant or inject active substances into a living organism.

In general, prior art collagen preparations for the active substance release are produced from acid-soluble collagen, that is collagen which is dissolved clearly in dilute acids at a pH of 2. This acid-soluble collagen can be isolated from several animal and vegetable tissues by means of a great variety of processes.

In contrast to this, the collagen used according to the present invention is an acid-insoluble collagen which - when it is present in aqueous dispersion - cannot be brought into solution even when concentrated acetic acid is added.
It is preferred that this insoluble collagen be native collagen the greater part of which is present as Type-I-collagen and the smaller part as Type-III-collagen. The term native collagen refers to a collagen molecule having an unchanged triple-helical tertiary structure.

The insoluble collagen used according to the present invention can be obtained from biomaterial of various origins by alkaline decomposition; for this purpose processes, such as those described, for example, in DE-OS 3034273, US 4 021 522, or DE-OS 27 16 602 are slightly modified. It is preferred that the starting material be calfskin, aged for six months. In contrast to the normally used tissues and body parts of cattle, pigs, or horses, this ensures a starting material of a defined, constant quality, this in turn ensuring the reliable reproducibility of the process steps described in the following and illustrated in greater detail in Example 1 hereunder.
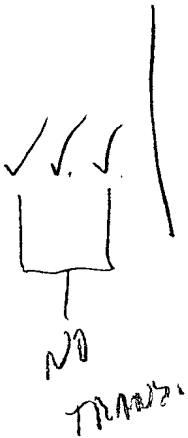
First, the calfskin is mechanically dehaired and degreased. Then, soluble collagens and non-collagenous soluble components of the connective tissue are extracted and rejected. Next, the connective tissue is first treated with an aqueous solution of an alkali hydroxide, preferably sodium hydroxide, and an alkali sulfate, preferably sodium sulfate, and then with an aqueous alkali sulfate solution to saponify and dissolve out sebaceous matter and to swell the collagen fibers in a controlled manner. When this is done, the terminal, non-helical portions of the collagen molecule, the so-called telopeptides which are mainly responsible for the antigenic properties of xenogeneic collagen, are also split off. In addition, the treatment with alkali hydroxide/alkali sulfate solution and with pure alkali sulfate solution splits a defined portion of the intermolecular collagen bonds in the fiber composite of the swollen connective tissue, said portion depending on the concentration and the duration of action of the solutions. The intramolecular bonds of the collagen are not attacked so that the helical structure of the molecule remains undamaged. The connective tissue so decomposed is washed out with water and dilute acids in several stages, purified and neutralized, and then mechanically comminuted and dispersed in water.

The decisive step for the isolation of the acid-insoluble collagen having different molecular weight distributions used according to the present invention is the selective alkaline cleavage of the intermolecular collagen bonds. If, for example, an aqueous solution of 9.75% sodium hydroxide and 9.2% sodium sulfate is allowed to react on the swollen connective tissue for 48 hours, the HPLC-analysis (column system Biosil TSK-400 + Biosil TSK-125; eluent 0.5 m ammonium acetate buffer pH 6.7; detector UV 275 nm; sensitivity range 0.02 AUFS) with assessment against standard-protein chromatograms shows an average molecular weight of about 420,000 for the insoluble collagen after dispersion in water. In contrast to this, if the swollen connective tissue is treated with

an aqueous solution of only 5% sodium hydroxide and 9.2% sodium sulfate for only 12 hours, far fewer intermolecular bonds are split, and the analysis of the insoluble molecular aggregates obtained after dispersion in water shows a mean molecular weight of about 2,500,000. These differences first of all affect the flow behavior and with that the processibility of the dispersions of insoluble collagen. Whereas a dispersion with 5% of low-molecular, insoluble collagen is a viscous but still free flowing mass, the flow limit of a dispersion of higher-molecular, insoluble collagen is at about 2.5%.

Thus, the variable factors of the decomposition process, by variation of which different molecular weight distributions can be achieved each time, are concentration and reaction time of sodium hydroxide. The higher the concentration of sodium hydroxide and/or the longer its duration of action, the lower the mean molecular weight of the isolated, acid-insoluble collagen, and vice versa. To represent the connection between the molecular weight that can be achieved and the described decomposition conditions, e.g., in the form of a curve, one of the influencing factors, i.e., concentration or duration of action, would have to be kept constant while the other one is changed. This is possible in principle, but from the production and operational point of view this is rather useless, since, for example, a free variation of the duration of action alone is not possible in general. For instance, very long reaction times with a correspondingly low sodium hydroxide concentration would increase both the machine running times and the requirement of personal; in view of the operating expenses this is not acceptable. On the other hand, owing to the required higher sodium hydroxide concentration, very short durations of action result in increased wear of the manufacturing facilities, e.g., of the conduits and filtering installation, and this cannot be realized either because of increasing working expenses. Therefore, the required duration of action and concentration of sodium hydroxide must

individually be ascertained empirically for each problem in such as manner that they represent an optimum both with respect to the demands on the administration form, in particular regarding the release kinetics of the active substance, and to economic efficiency.

The differences in the molecular weight distribution of insoluble collagen distinctly influence the properties of the collagen preparations that are produced therefrom by means of drying. Example 2 shows that, depending on the mixing ratio, foams of insoluble collagen with different molecular weight distributions produced by means of freeze-drying have very different disintegration properties. While foams consisting of 100% of low-molecular, insoluble collagen completely decompose in artificial wound exudation of pH 6.4 after only 45 minutes, foams made of 100% of higher-molecular, insoluble collagen have not disintegrated even after 10 days under the same conditions.

The example shows that the use of high-molecular collagen aggregates has a clearly stabilizing effect on the foam; this is expressed in a clearly slowed down absorption of secretion and swelling, and in a very delayed disintegration. Form stabilization by means of maintaining a high natural cross-linking degree of the collagen has the important advantage, in particular from the toxicological point of view, that no additional manipulations to consolidate the structure, e.g., by tanning or cross-linking, are required. The product is rendered dimensionally stable by merely maintaining the collagen's original quaternary structure, such as that present in the skin, to a substantial degree.

Thus, the collagen foams, each manufactured from only one single basic type of insoluble collagen, show clear differences, for example, with respect to interaction with the secretion of the wound. However, as mentioned above, the profile which is

required for a collagen product and is based on a specific purpose can only in special cases be satisfied by such mono-preparations. Compared with that, the present invention offers two possibilities of exactly tailoring the properties of a collagen preparation to given demands on a product. On the one hand, the molecular weight distribution of the insoluble collagen can continuously be varied over a very wide range by selectively controlling the cleavage of the intermolecular bonds. On the other hand, the required product properties can be adjusted by mixing said variations of insoluble collagen each having different molecular weight distributions. If the already mentioned Example 2 is continued, this becomes clear, for instance, by the disintegration of freeze-dried foams, where - prior to drying - insoluble collagen having a mean molecular weight of about 420,000 and insoluble collagen having a mean molecular weight of about 2,500,000 were mixed at always different ratios. With different pH-values, the decay periods always show a gradual decrease when the percentage of insoluble collagen having a low average molecular weight is gradually increased.

The exact adjustment of the product properties to a demand profile representing the optimum for the respective therapy is of importance primarily in the therapy using pharmaceutical active substances.

Since the collagen preparation according to the present invention is preferably used on the skin, on external and internal wounds, and in internal body tissues and body cavities after implantation or injection, the active substances suitable for charging the collagen preparation preferably are active substances for the dermal and transdermal application, active substances for the treatment of wounds and for the promotion of wound-healing, as well as active substances usually administered by means of preparations for implantation or injection.

For the dermal treatment of local skin diseases the following sub-
stances are used: local anaesthetics, local antibiotics, antiseptics,
antimycotics, antihistaminics, and antipruritic drugs; keratolytics
and caustic drugs; virustatics, antiscabietic agents, steroids, as
well as different substances for the treatment of acne, psoriasis,
or photodermatoses. Active substances applied intradermally in-
clude, for example, steroid and non-steroid antirheumatics, sub-
stances stimulating the blood flow, or vasoprotectors and vaso-
constrictors for the treatment of vascular diseases. The active
substances applied transdermally include, for example, neurolep-
tics, antidepressants, tranquilizers, hypnotics, psychostimulants,
analgesics, muscle relaxants, antiparkinson drugs, ganglionic
blockers, sympathomimetics, alpha-sympatholytics, beta-sym-
patholytics, antisympathotonics, antidiabetics, coronary therapeu-
tic agents, antihypertensives, anti-asthmatics, or diuretics. The
collagen preparation according to the present invention can also
be used on the skin in cosmetic preparations, e.g., in the form of
lather masks or films for the treatment of, for example, aged skin,
wrinkles, or impure skin; for body care, depilation, reduction of
perspiration, or for light protection.

Active substances which are used in the collagen preparations ac-
cording to the present invention on external and internal wounds,
preferably are styptic active substances, among which collagen
itself has a special place; wound-cleansing substances, such as
enzymes, antiseptics, disinfectants, and antibiotics; as well as ac-
tive substances promoting wound healing which stimulate granu-
lation, induce vascularization, or promote epithelization. Among
the active substances promoting the wound healing, biologically
active peptides and proteins - which develop high activities at only
very low concentrations and are mainly manufactured by recombi-
nant technologies - increasingly gain importance. The collagen
preparation according to the present invention represents a par-
ticularly suitable carrier and release system for these substances

which include the so-called growth factors, such as Platelet de-
rived growth factor (PDGF), Epidermal growth factor (EGF), Plate-
let derived endothelial cell growth factor (PD-ECGF), acidic Fibro-
blast growth factor (aFGF), basic Fibroblast growth factor (bFGF),
Transforming growth factor α (TGF α), Transforming growth factor
β (TGF β), Keratinocyte growth factor (KGF), Insulin-like growth
factors 1 and 2 (IGF1, IGF2), and Tumor necrosis factor (TNF).

The active substances administered parenterally by means of the
collagen preparation according to the present invention include,
for example, antibiotics, antiseptics, anaesthetics, analgesics of
varying strengths; cytostatics, hormones, steroids, cytokinins,
such as interleukins, interferons, and colony-stimulating factors,
Hormone releasing and release inhibiting factors, prostaglandins,
enzymes, as well as growth factors, in particular osteoinductively
effective bony growth factors.

One of the greatest challenges for the development of active sub-
stance-containing preparations is to find formulations releasing the
active substance in such a manner that the optimum action and
best therapy is achieved. The half-life period of many active sub-
stances, in particular of the above-mentioned biologically active
peptides and proteins, in the body is relatively short, and for this
reason they must frequently be administered several times a day.

Therefore, active substance vehicles releasing the active sub-
stances in a delayed or even controlled manner gain increasing
importance. Collagen preparations according to the present inven-
tion offer the possibility of developing carrier systems using rela-
tively few formulation base components. These systems are very
flexible both with regard to application and design, and can ex-
actly be directed to the required solution of a problem. The
mechanisms, which impart to the release kinetics of an active
substance its characteristic features, can selectively be controlled

by mixing of insoluble collagen having different molecular weights and by shaping of the collagen preparation. These factors, in addition to structure and density of the polymeric collagen skeleton, also influence the number and distribution of hydrophilic, hydrophobic and ionic bonds along the polymer skeleton. Since active substance can not only be enclosed in cavities of a collagen preparation according to the present invention, but can also be adsorbed to the surface of the collagen skeleton, the bonding force between collagen and active substance - and thus its release behavior - is substantially determined by ionic relations as well as by hydrophilic and hydrophobic interactions.

In the dermal, intradermal and transdermal application of an active substance using a collagen preparation according to the present invention, the solubility of the active substance in the preparation, the degree of charge and saturation, and the diffusion rate of active substance within the preparation - in addition to structure, density and bonding activity of the collagen preparation - have an influence on the release behavior.

In case of collagen preparations for the release of active substances to external or internal wounds and collagen preparations for implantation or injection another factor appears.

Since the collagen preparations according to the present invention come into contact with body fluid in the applications mentioned above, the rate and amount of liquid absorbed by the collagen preparation and consequently the swelling capacity and disintegration properties of the collagen preparation can be used to control the release, as is shown in Example 2 mentioned hereinbefore. In addition to the decay of the collagen preparation used as controlling mechanism, other suitable mechanisms with regard to the release control on contact with body fluid primarily include the dissolution of active substance out of the collagen preparation by

means of body fluid and the fluid-induced diffusion of active sub-
stance from the center of the collagen preparation to its interface.
Furthermore, the release is influenced by the biological degrada-
tion of the collagen preparation by means of hydrolysis and enzy-
matic reaction on contact with body fluid. The wide range of in-
teraction between the liquid and preparations of mixtures of in-
soluble collagen having different molecular weight distributions
has already been shown in Example 2. The way said interactions
in combination with one or several of the above-mentioned influ-
encing factors take effect on the release kinetics of an active sub-
stance is illustrated Examples 3 and 4. It is made clear that the
mixing ratio of insoluble collagen having different molecular weight
distributions significantly influences the kinetics of the active sub-
stance release. In the case of a given active substance and a given
desired release per time unit, it is thus possible to adapt the colla-
gen preparation selectively to the required demands; in this way
an optimum active substance dosage for the respective therapy is
achieved after application over the pre-determined application pe-
riod. If active substance is to be released over an application pe-
riod of 24 to 48 hours from the administration form chosen in Ex-
amples 3 and 4 - manufactured of the preparations of insoluble
collagen having different molecular weight distributions as de-
scribed in Example 1 - the mixing ratio of low-molecular and high-
molecular insoluble collagen should not be lower than 3 to 1. If,
on the other hand, an even, delaying active substance release
from a comparable administration form is to take place over an
application period of 7 to 14 days, the mixing ratio of low-molecu-
lar and high-molecular insoluble collagen should not be greater
than 1 to 3.

In order to prove the controlling possibilities of preparations con-
sisting of mixtures of insoluble collagen having different molecular
weight distributions, free from any influence of other auxiliary
agents, pure collagen preparations plus active substance were

exclusively used in the Examples on purpose. In practice, how-
ever, it will not be possible to do without additional water-soluble
or water-dispersible additives, since, in addition to the require-
ments regarding the active substance release, the intended pur-
pose will usually also result in demands with respect to handling
properties and stability of an active substance-collagen-prepara-
tion.

Such adjuvants may be additional polymers, e.g., serving as vis-
cosity regulators when liquid preparations are used or as binders
when solid forms are used, for example, cellulose derivatives,
starch derivatives, galactomannan derivatives, dextrans; vegetable
polysaccharides, such as alginates, pectins, carrageenan, or xan-
than, chitosan, proteins, glycoproteins, proteoglycans, glucosami-
noglycans, polyvinyl alcohol, polyvinylpyrrolidone, vinyl pyrroli-
done-vinyl acetate copolymers, polyethylene glycol, polyacrylates
and polymethacrylates, polylactides and polyglycolides, as well as
polyamino acids.

The collagen preparation may comprise as additional adjuvants:

- humectants, such as glycerol, sorbitol, polyethylene glycol,
   polypropylene glycol,

- softening agents, such as citric acid ester, tartaric acid ester,
   or glycerol ester,

- penetration enhancers, such as alkyl sulfates, alkyl sulfonates,
   alkali soaps, fatty acid salts of multivalent metals, betaines,
   amine oxides, fatty acid esters, mono-, di- or triglycerides,
   long-chain alcohols, sulfoxides, nicotinic acid ester, salicylic
   acid, N-methyl pyrrolidone, 2-pyrrolidone, or urea,

- preservatives, such as p-Cl-m-cresol, phenylethyl alcohol, phe-
noxyethyl alcohol, chlorobutanol, 4-hydroxybenzoic acid meth-
ylester, 4-hydroxybenzoic acid propylester, benzalkonium chlo-
ride, cetylpyridinium chloride, chlorohexidine diacetate or diglu-
conate, ethanol, or propylene glycol.

- Disinfectants, for example, halogens, such as polyvidone-
iodine; halogen compounds, such as sodium hypochloride or
tosylchloride sodium; oxidants, such as hydrogen peroxide or
potassium permanganate; aryl mercury compounds, such as
phenylmercury borate or merbromin; alkyl mercury compounds,
such as thiomersal; organotin compounds, such as tri-n-butyl-
tin benzoate; silverwhite compounds, such as silverwhite ace-
tyltannate; alcohols, such as ethanol, n-propanol, or isopro-
panol; phenols, such as thymol, o-phenylphenol, 2-benzyl-4-
chlorophenol, hexachlorophene, or hexylresorcinol; or organic
nitrogen compounds, such as 8-hydroxyquinoline, chloroqui-
naldol, clioquinol, ethacridine, hexetidine, chlorohexidine, or
ambazone.

- pH-regulators, such as glycerol buffers, citrate buffers, borate
buffers, phosphate buffers, or citric acid phosphate buffers.

- Antioxidants, such as ascorbic acid, ascorbyl palmitate, toco-
pherol acetate, propyl gallate, butylhydroxyanisole, or buty-
lated hydroxytoluene.

- Active substance stabilizers, such as mannitol, glucose, lac-
tose, fructose, saccharose,

- emulsifyable inactive ingredients, such as oils, fats, and
waxes,

- odorous substances, dyes, cleaning agents, substances for personal hygiene,

- emulsion stabilizers, such as non-ionogenic emulsifiers, ampho- teric emulsifiers, cation-active emulsifiers, and anion-active emulsifiers,

- fillers, such as micro-crystalline cellulose, aluminum oxide, zinc oxide, titanium oxide, talcum, silicon dioxide, magnesium sili- cate, magnesium aluminum silicate, kaolin, hydrophobic starch, calcium stearate, or calcium phosphate.

For example, adjuvants may be dissolved, dispersed, or emulsified in dispersions of insoluble collagen prior to shaping the admini- stration form. However, they may also be introduced in a manner usual and known for the respective shaping processes after termi- nation of a primary forming process (described hereinafter) of the mixtures of insoluble collagen during later stages of the drug shaping.

The embodiments wherein admixtures of insoluble collagen of dif- ferent molecular weight distributions may be used for the con- trolled release of active substances are so numerous and manifold that they cannot be represented herein extensively.
These embodiments of the collagen preparation according to the present invention may be manufactured from mixtures of disper- sions of insoluble collagen each having different molecular weight distributions according to various methods known to the skilled artisan; for example by spray drying, freeze-drying, coating or casting with subsequent drying, phase separation and coacerva- tion processes for particles or emulsified droplets, drying and com- pression, as well as by simple filling into containers, e.g., tubes. They result in, for example, powders, dusts, microparticles, fibers, flakes, foams, sponges, needles, small rods, tablets, gels, creams,

single-layer films, or laminates. Preferred embodiments are spray-dried microparticles, freeze-dried foams, and gel-like films manufactured by coating.

The introduction of active substance into the collagen preparation according to the present invention may be effected such that the active substance is dissolved or dispersed in the finished mixture of dispersions of insoluble collagen having different molecular weight distributions, prior to forming the preparation. If more than one active substance is to be incorporated, these may also be dissolved or dispersed separately from one other in the individual fractions of insoluble collagen, prior to the mixing process. Active substance may also be introduced on or into the preparation after the shaping process of the preparation by means of coating, spraying, impregnating, dipping, or other adsorption methods.

The possible embodiments, the respective manufacturing processes, and the methods of incorporating the active substance may also be combined with one another in order to achieve certain properties.

For instance, the collagen preparation according to the present invention may comprise a shell of insoluble collagen with a low mean molecular weight, which is soluble or at least swellable in body fluid, in the form of a sponge or film containing a micro-particulate preparation of insoluble collagen having a high mean molecular weight dispersed therein. If only one active substance is incorporated, the outer sponge or film phase serves the quick release of active substance to achieve the required minimum active substance concentration very quickly, followed by a slower, more even release of active substance from the inner, particulate phase to maintain the required active substance concentration over the application period. Such release profiles may also be achieved with other preparation forms, such as fibers in hydrogels, spongy

oil-in-water-emulsions, compressed mixtures for implantable tab-
lets, multi-layer films, combinations of films and foams, and the
like.

Such multi-phase preparations can also be used if a release of
different active substances at different points of time with differ-
ent release rates is desired, or if one or several active substances
are to be released phase-like, i.e., with release-free intervals.
In this case active substance A may, for example, be contained in
a readily soluble, quick-releasing outer phase, whereas active
substance B is contained in an inner phase which is slightly solu-
ble and releases the active substances in a retarded and controlled
sustained manner. If a phase-like release profile is desired for only
one active substance, external and internal phase may, for exam-
ple, release active substance at an even kinetics. However, the
inner phase will then be surrounded by an active substance-free
layer of insoluble collagen, which, after dissolution of the outer
phase or active-substance-exhaustion thereof, must swell or dis-
solve itself first so that the active substance release from the inner
phase can take place. By means of this arrangement, an interval
without any active substance release can be obtained.

If the collagen preparation according to the present invention is
used for the dermal, intradermal or transdermal application of
pharmaceutical active substances or cosmetic active principles,
plane embodiments, such as films, membranes, or thin sponges
are preferred. These flat embodiments may consist of laminates
which, for example, also include barrier layers which are free from
active substances, permeable separating layers, controlling mem-
branes, and adhesive layers. Substructures, such as laminae,
powders, microparticles, or oil droplets, may then be introduced in
or between the individual layers, in order to achieve the suitable
release kinetics for one or several active substances. It is preferred
that such single- or multi-layer collagen preparations for the

mentioned applications, e.g., for protection against dehydration or growing in of germs, be provided with a backing layer and a removable protective layer located on the opposite side according to known processes, the backing layer and the protective layer consisting of materials known to the skilled artisan, for example, those used in the formulation of patches and adhesive tapes.

In a preferred embodiment of the collagen preparation according to the present invention for the use on external wounds and in the interior of the body, the preparation is paste-like, e.g., foamy or spongy. The size of the pores and the structure of the preparation are designed such that immigration of cells, e.g., fibroblasts or osteoblasts, into the preparation is possible and that the cells are given a structural orientation; this can particularly be attributed to the degree of orientation of the collagen in the preparation according to the present invention, which is similar to that of natural connective tissue. Immigration of the cells may be necessary, for example, for the degradation of the preparation or for the release or deposition of substances which are required, for example, for neo-formation of tissue or vascularization of a tissue which is to take the place of the collagen preparation according to the present invention.

In another preferred embodiment for the use on wounds or in the interior of the body, the collagen preparation according to the present invention is adjusted by admixing adjuvants, such as carboxymethylcellulose, polyacrylic acid, tragacanth, sodium alginate, or hydroxypropylcellulose in such a manner that it is bioadhesive, i.e., that it adheres to the surface tissue of the application site for a certain time by means of interfacial forces, in order to increase the retention time at the site of application or absorption.

Examples

1. Extraction of insoluble collagen from calfskin

1.1 Treatment with $Ca(OH)_2$ 1% in H_2O 100 h
Treatment with NH_4Cl 3% in H_2O 1 h
Treatment with NaOH 5% + $Na_2 SO_4$ 9.2% in H_2O 12 h
Treatment with $Na_2 SO_4$ 1 molar in H_2O 0.5 h
Treatment with HCl 1% in H_2O 1.5 h
Treatment with H_2O_2 0.5% in H_2O 6 h

After mechanical comminution and dispersion in H_2O (pH 6.0) the insoluble collagen has an average molecular weight of about 2,500,000. The collagen concentration amounts to 0.75%.

1.2 As in a), difference:

Treatment with $Ca(OH)_2$ 1% in H_2O 72 h
Treatment with NaOH 9.75% + $Na_2 SO_4$ 9.2% in H_2O 48 h

After mechanical comminution and dispersion in H_2O (pH 6.0) the insoluble collagen has an average molecular weight of about 420,000. The collagen concentration amounts to 0.75%.

2. Production of active substance-free lyophilizates from mixtures of the dispersions according to 1.1 and 1.2 and determination of the disintegration time in buffer mixtures having different pH-values.

The following mixtures of the dispersions according to 1.1 and 1.2 were produced:

Dispersion 1.1		Dispersion 1.2
A	100 %	0 %
B	75%	25%
C	50%	50%
D	25%	75%
E	0%	100%

24 g of the mixtures A to E were each filled into deep-drawing dies of 6 x 4 x 1.5 cm, shock-frozen and brought to a temperature of -50°C within 60 minutes. Subsequently the mixtures were freeze-dried.

The disintegration time of the resulting sponges (dimensions 6 x 4 x 0.8 cm) was determined under slight stirring in 100 ml of the following buffer mixtures:

pH 3:   buffer mixture citric acid/sodium chloride/sodium hydroxide solution with addition of a fungicide

pH 5:   acetic acid (0.1 molar) sodium acetate (0.2 molar)

pH 6.4: artificial exudation of a wound (without albumin)

pH 7.5: potassium hydrogenphosphate/NaOH (0.1 molar/0.1 molar)

Results:

Mixture	pH of buffer solution			
	3	5	6.4	7.5
A	2 h 15 min	> 10 d	> 10 d	> 10 d
B	1 h 10 min	3 d	3 d	2 d
C	1 h	24 h	24 h	6 h
D	20 min	4 h 10 min	4 h 30 min	1h 45min
E	2 min	35 min	45 min	15 min

3. **Production of lyophilizates with p – hydroxybenzoic acid propylester and determination of the release**

0.275% (relative to the dispersion) of p-hydroxybenzoic acid propylester were dissolved in each of the mixtures A to E according to Example 2. 10 g of each of the mixtures were filled into deep-drawing dies according to Example 2, shock-frozen, cooled to -50°C within 60 mins., and then freeze-dried.

About 30 mg (theoretical PHB-ester-content 6.1 mg) of each of the resulting lyophilizates (sponge weights about 135 mg, theoretical PHB-ester-content 27.5 mg) were placed in a paddle-device and stirred at 45 rpm in 500 ml of 0.05 N sodium hydroxide solution at 37°C. After 4 hours, 10 ml of each release medium were withdrawn. The active substance content was determined by ultraviolet-photometry against standard at 294 nm at a layer thickness of 1 cm.

Results:

Mixture	A	B	C	D	E
released act. subst. (mg)	2.1	2.7	3.4	3.8	*

*could not be determined because of complete disintegration of lyophilizate

4. **Production of lyophilizates with lidocaine hydrochloride and determination of the release**

0.042% (relative to the dispersion) of lidocaine hydrochloride were dissolved in each of mixtures A to E according to Example 2. 24 g of each mixture were filled into deep-drawing dies according to Example 2, shock-frozen, cooled to -50°C

within 60 minutes, and then freeze-dried.

The resulting lyophilizates (sponge weights about 250 mg, theoretical content of lidocaine hydrochloride 10 mg) were each filled into a paddle-device and fixed at the bottom of the vessel by means of a net. 350 ml of water having a temperature of 37°C were filled into the device and stirred at 45 rpm. After 24 hours, samples of the release medium were taken. The active substance content was determined at 262.5 nm and a layer thickness of 1 cm by means of ultraviolet- photometry and assessment based on a standard curve.

Results:

Mixture	Active substance released
A	1.2 mg
B	5.1 mg
C	7.4 mg
D	8.8 mg
E	10.0 mg